



An update on research advances in rheumatoid arthritis: from clinic to basic science

Elisa Corsiero¹, Alessandra Marrelli²

¹Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, UK; ²Department of Medicine, Geriatric Unit, Immunology Clinic, San Salvatore Hospital, L'Aquila, Italy

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Correspondence to: Elisa Corsiero. Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London EC1M 6BQ, UK. Email: e.corsiero@qmul.ac.uk; Alessandra Marrelli. Department of Medicine, Geriatric Unit, Immunology Clinic, San Salvatore Hospital, L'Aquila, Italy. Email: alessandramarrelli@tiscali.it.

Abstract: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterised by breach of self-tolerance and production of autoantibodies, particularly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). Many events can lead to loss of tolerance in RA and they include genetic, environmental and epigenetic factors as well as post-translational modifications. Current therapies in RA tend to treat symptoms or slow down the disease progression but no cure exists at the moment. This review provides a summary of the main recent research advances regarding new therapies in RA and new studies about the effect of genetic, environment and epigenetics in this autoimmune disease. Moreover, it summarises the present knowledge about the role of “new” autoantibodies in RA focussing on anti-carbamylated protein antibodies (anti-CarP), anti-hinge antibodies, anti-acetylated protein antibodies and anti-malondialdehyde-acetaldehyde adducts (MAA) antibody. Finally, a brief summary on the recent role of Fab N-glycosylation is reported.

Keywords: Rheumatoid arthritis (RA); pathophysiology; anti-modified protein antibodies (AMPAs); therapy

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Introduction

Rheumatoid arthritis (RA) is the most common inflammatory erosive polyarthritis affecting around 0.5–1% of the worldwide population which leads to joint damage and disability. RA is also associated with systemic complication including cardiovascular, pulmonary, psychological, skeletal disorders, and early death (1,2). In addition, RA is associated with socioeconomic costs derived from medical costs as well as from functional and social disability (3). RA is characterised by breach of self-tolerance and production of autoantibodies. Many factors including susceptibility genes, environmental factors, epigenetic

modifications and post-translational modification can lead to loss of tolerance. The main-known division in RA is between patients characterised by the presence of anti-citrullinated peptide/protein antibodies (ACPA-positive) and patients without ACPA (ACPA-negative). In this review, we focus on recent research advances of RA covering novel therapies and pathogenesis.

Therapy in RA: recent advances

RA is a chronic autoimmune disease characterized by inflammation and deterioration of the joints which can produce a loss of functionality, reduces quality of life

and enhances morbidity and mortality. The main goal of RA treatment is to stop inflammation early during the disease course, relieve symptoms, prevent joint and organ damage, improve physical function and reduce long-term complications (4).

To meet these goals, current treatment models promote to start an early aggressive approach, to target remission and to maintain a tight control. The strategy of early treatment initiation is strongly supported by the concept of “window of opportunity” which presumes a starting phase of RA when the disease is less matured, resulting most susceptible to the disease modifying effect of the treatment (5,6). In order to facilitate the understanding of this therapeutic “window of opportunity” the European League Against Rheumatism (EULAR) study group has outlined several risk factors for RA defining several stages: genetic and environmental risk factors for RA, systemic autoimmunity associated with RA, symptoms (arthralgia) without clinical arthritis and clinical arthritis either unclassified arthritis (UA) and RA (7).

Over the last 20 years, the effectiveness of disease-modifying anti-rheumatic drugs (DMARDs) in RA has gained much attention as these can efficiently attenuate disease activity and substantially decrease and/or delay joint deformity. The therapy classification includes the traditional synthetic drugs, biological DMARDs, and novel potential small molecules. At present, all evidence supporting early treatment initiation come from studies of patients with clinically manifest arthritis. Very few trials on treatment initiated in the pre-arthritis phases have been published to date.

A systematic literature review on murine studies suggested that DMARD initiation in the induction phase of experimental arthritis, prior to clinical arthritis, was effective in controlling disease severity. Moreover, the authors of this study found that the treatment was also effective in the setting in which autoimmunity has developed without clinically evident arthritis (8).

The first placebo controlled trial assessing the effect of treatment during the first pre-clinical stages of the disease was published in 2009 and showed that dexamethasone reduced autoantibodies level in RA patients with ACPA-positive and/or rheumatoid factor (RF)-positive arthralgia, without preventing the development of arthritis (9).

Recently, the PRAIRI (prevention of clinically manifest RA by B cell directed therapy in the earliest phase of the disease) trial showed that a single infusion of rituximab in patients with ACPA-positive and RF-positive arthralgia with

C reactive protein (CRP) levels ≥ 3 mg/L and/or subclinical synovitis on ultrasound (US) or MRI of the hands, delayed the onset but did not prevent the development of clinical arthritis (10). In a different study, treatment of RA patients with rituximab was shown to decrease the levels of IgA-RF, IgG-RF, and IgG ACPAs significantly more than the total serum Ig of the same isotypes (11). Results of a randomized, double-blind, placebo-controlled study of the potential of a single rituximab infusion in the prevention of RA in high-risk arthralgia patients were described in a 2016 EULAR conference abstract. All 81 treated patients were RF and ACPA positive and received 100 mg methylprednisolone with 1,000 mg rituximab or placebo. The rituximab did not prevent but delayed the development of arthritis by nearly 1 year (12). The cornerstone of this “pathobiology driven approach” is to identify with accuracy and confidence the predictors of RA development during its preclinical stages to reach better clinical and therapeutic outcomes. The importance of including patients with a high risk of progression to RA was emphasized in the Probable RA: Methotrexate versus Placebo Treatment (PROMPT) trial, in which patients with UA were treated with methotrexate with the aim of evaluating RA development. The risk of progression to RA was ~30%, and without further stratification, methotrexate did not modify this risk. However, when only patients with a high (>80%) 1-year predicted risk of progression to RA were evaluated, methotrexate was highly effective in preventing progression to RA. In addition, methotrexate was also associated with DMARD-free remission in this high-risk group (36% *vs.* 0% in the placebo group) (13).

Investigation to identify predictive tools for RA development is a highly active and prolific area of research. Furthermore, RA has heterogeneous etiology and pathophysiology, thus progression to RA is not easily recognized. In the absence of pathognomonic markers, multiple biomarkers should be combined to predict patients which will progress to RA.

Autoantibodies play an important role during the developing phase of autoimmunity. Although, no definitive causal link with the development of arthritis has been proved, they are part of American College of Rheumatology (ACR)/EULAR RA classification and are recognized as important risk factors for future RA (14). The presence of autoantibodies does not always lead to development of RA; this may be explained by the heterogeneous character of the various autoantibody responses that can be present in individuals at risk for RA, with different intrinsic properties

such as affinity, specificity, isotype composition, and glycosylation as discussed later in this review. Moreover, autoantibody responses can change and evolve into more pathogenetic properties during the first phase of the disease leading up to the clinical manifestations of autoimmunity.

Genetic and epigenetic: the new insights

The genetic component plays an important role in the pathogenesis of RA. The combination of large clinical cohorts and progresses in genetic technologies have enhanced our knowledge on the genetic aspect of RA. Up to date, more than 100 loci have been associated with RA. The human leucocyte antigen (HLA)-DRB1 locus, one of the oldest to have been identified, is strongly associated with RA risk, and in particular HLA-DRB1*01, *04 and *10 alleles are correlated with a high risk of developing the disease in ACPA-positive patients (15,16). These HLA-DRB1 alleles share in the peptide-binding groove an identical amino acid sequence, also known as shared epitope (SE). Since the correlation with ACPA-positive patients is high, it has been suggested that the peptides presented by the SE alleles may be citrullinated (16). Recent studies in the Southern Indian population have added new insights into the HLA-DRB1 locus showing that HLA-DRB1*03, *07, *11 and *13 alleles are associated instead with a protective role in the disease (15,17). In addition to HLA-DRB1, HLA-DRB4 locus has been associated with RA, mostly in correlation with prediction to response to methotrexate in early RA (15,18). Furthermore, other non-HLA loci has been recently characterised. Among the non-HLA genes, single nucleotide polymorphisms (SNPs) in the signalling transducers and activators of transcription (STAT)-4 and interleukin (IL)-10 genes seem to be correlated with the disease (15). In particular, in a cohort of Italian RA patients Ciccacci *et al.* showed that the rs7574865 in the *STAT-4* gene was associated with a higher risk of developing RA, whereas the rs1800872 in *IL-10* gene was related to a protective outcome (19). Moreover, rs7574865 in *STAT-4* and rs1800872 in *IL-10* were associated with presence of ACPA and RF, respectively. Additionally, they showed that SNPs in psoriasis susceptibility 1 candidate 1 (PSORS1C1), protein tyrosine phosphatase, non-receptor type 2 (PTPN2), and microRNA (MIR)146A were associated with a strong disease phenotype in term of autoantibody production and joint damage (19). Besides these non-HLA loci, mutations in the protein tyrosine phosphatase, non-receptor type (PTPN)-22 gene are strongly associated with the risk of developing RA.

This gene encodes a protein tyrosine phosphatase involved in the signalling pathway in lymphoid cells. Recently, it has been shown that mutations in this gene are associated with hypercitrullination of peripheral blood mononuclear cells through the activity of peptidyl arginine deaminase (20) enzymes (21,22).

Besides genetics, epigenetic mechanisms which include DNA methylation, histone modification and microRNAs also contribute to the RA pathogenesis. Using a comprehensive genome-wide methylation analysis, Liu *et al.* (20) recently showed the presence of ten differentially methylated sites that could be associated with the risk of developing RA. Moreover, current studies have started to combine high risk genetic loci analysis with epigenetic regulation (15). For instance, a recent study on RA fibroblast-like synoviocytes (FLS) function has showed a link between a characteristic DNA methylation signature and dysregulation of genes like PTPN11 (23). In particular, in this work the authors studied the presence of regulatory regions in PTPN11 and RA-specific epigenetic changes showing that an intronic glucocorticoid receptor-responsive enhancer in this gene, which is very activated in RA-FLS, is hyper-methylated and this is necessary for full enhancer activity. Finally, Gaur *et al.* recently showed that microRNAs might selectively target DNA methylation mechanisms in RA-FLS (24).

The growing importance of microbiota

The pathways leading to RA is associated not only with susceptibility genes and epigenetic modifications. An additional component is represented by environmental effects including smoking, exposure to infectious agents such as *Escherichia coli* and Epstein-Barr virus, and microbiome (25). In particular, the effect of the microbiome (both gut and oral) on RA disease risk and progression has become the subject of several studies. The composition of the gut microbiota has been shown to be altered in patients with RA and this has been found to aggravate or alleviate arthritis rather than induce the disease (15,26,27). A recent study has demonstrated that RA patients have a decreased gut microbial diversity compared to healthy individuals and this was correlated with autoantibody levels and disease duration (26). In this work, the authors showed that RA is characterised by the expansion of certain rare bacteria, particularly *Eggerthella*, *Faecalibacterium*, and *Collinsella*, and that the latter was strongly correlated with the production of pro-inflammatory cytokines such IL-17A.

Porphyromonas gingivalis, a bacterium found in periodontitis, has been also associated with RA since through the expression of its own peptidylarginine deiminases (PAD) it can support aberrant citrullination leading to breach of tolerance towards citrullinated peptides (28). Besides *P. gingivalis*, recent studies have identified another bacterium (*Aggregatibacter actinomycetemcomitans*) which can potentially dysregulate citrullination by human PAD enzyme bringing to endocitrullination in gingival crevicular fluid (GCF) of patients with periodontal disease (29). This bacterium can produce pore-forming toxin leukotoxin A (LtxA) inducing an increase of calcium influx into neutrophils leading to hypercitrullination via the cells' own PAD enzymes. Patients with RA are characterised by anti-LtxA antibodies which has been shown to be significantly associated with the presence of ACPA and RF (16,29). Thus, a better understanding on how environmental factors can influence the immune response in RA is important in order to clarify their contribution to the disease development.

ACPA in RA

The presence of ACPA is associated with more severe RA and articular destruction (30,31). Several studies have outlined the involvement of ACPA in the pathogenesis of RA. Results from animal studies demonstrated arthritogenicity of some types of ACPA; in fact, anti-citrullinated fibrinogen antibodies and anti-citrullinated collagen antibodies bound targets within the inflamed synovium and enhance tissue injury in murine experimental arthritis (32,33). Sokolove *et al.* demonstrated that complexes consisted of citrullinated fibrinogen and ACPA (CitFibr-ACPA) present in RA synovium can stimulate macrophages leading to induction of TNF- α production by human macrophages (34). Induction of TNF- α could be further amplified by IgM-RF and extended to the secretion of other pro-inflammatory cytokines (IL-1 β , IL-6 and IL-8) that activate RA synoviocytes (35).

Recently, another ACPA-mediated mechanism of TNF- α induction that may operate in RA has been described. Through binding to surface, over-expressed citrullinated glucose-regulated protein 78 on RA peripheral blood mononuclear cells, ACPAs selectively activate ERK1/2 and JNK signalling pathways to enhance IKK- α phosphorylation, which leads to the activation of NF- κ B and the production of TNF- α (36).

Pathogenic activity of ACPA in RA is also associated with induction of NETosis, a specific type of cellular death that

consists in the extrusion of the intracellular material (DNA, histones, granular proteins and cytoplasmic proteins) resulting in creation of neutrophil cellular trap—neutrophil extracellular trap (NET) by neutrophils. Anti-citrullinated vimentin antibodies were shown to potently induce NET formation. Presence of NET augments further the activities of synovial fibroblasts, which secrete pro-inflammatory cytokines, chemokines and upregulate adhesion molecules. Pro-inflammatory cytokines are in turn the stimulus for NET formation. Furthermore, accelerated NETosis in RA is a source of citrullinated autoantigens (including vimentin and histones), and PAD enzymes that when released from intracellular compartment can citrullinate extracellular proteins (37,38), further fuelling ACPA production. Hence, stimulation of NET formation by ACPA may perpetuate the inflammation and autoimmunization processes in RA.

In vitro and *in vivo* studies showed also that ACPA contribute to bone destruction. ACPA bound to the surface of osteoclasts and osteoclast precursor cells and induced their differentiation as well as activated bone-resorption activity. Transfer of ACPA derived from RA patients into mice, caused arthralgia and systemic bone loss before signs of joint inflammation appeared (39,40). Stimulation of osteoclastogenesis by ACPA relied on inducible autocrine secretion of pro-inflammatory cytokines (TNF- α , IL-8) by osteoclast precursor cells.

The growing importance of ACPA Fab glycosylation in RA

ACPA are glycoproteins where carbohydrate chains (or glycans) are attached to both the Fc and Fab domain of the antibody. N-Glycosylation sites are conserved region formed by asparagine (N), followed by any amino acid but not proline (X), and either serine/threonine (N-X-S/T). For many years, most studies have been focused on N-linked glycans in the Fc domain. In particular, it has been shown that ACPA have a lower level of galactosylation and sialylation in the Fc domain compared to IgG from healthy donors (41). Interestingly, it has been shown that less Fc-sialylation can drive *in vitro* and *in vivo* osteoclastogenesis due to a different Fc R signalling pathway activation and that patients with a reduced ACPA Fc-sialylation have lower bone volume and trabecula numbers (42). In the last few years, Fab N-linked glycosylation of ACPA has gained interest. In 2015, Rombouts and colleagues (43) demonstrated that ACPA have a higher molecular weight compared to IgG from healthy donors due to an increase

of Fab glycans. ACPA Fab glycans have more galactose, sialic acid and fucose residues compared to controls (44). It is still unclear why ACPA Fab glycans differ from ACPA Fc glycans but it has been proposed that environmental factors as cytokines might have a role in determining the glycans composition (16). Recently, it has been proposed that Fab glycosylation might influence antigen binding or the half-life of ACPA (44). However, more studies need to be performed in order to dissect deeply the role of Fab glycosylation on ACPA.

Autoantibodies in RA: beyond ACPA

RF and ACPA are the two main diagnostic markers for RA included in the ACR/EULAR 2010 classification criteria (45). In particular, ACPA which were first described in 1964 (46) have been extensively studied with the identification of a wide array of citrullinated proteins as target of ACPA (e.g., vimentin, alpha-enolase, fibrinogen, histones). Citrullination is a post-translational modification mediated by PAD enzymes. As mentioned before, the presence or absence of ACPA allow the identification of subgroups of RA patients. Recently, other post-translational modifications have started to gain interest in RA giving rise to a group of AMPAs. Here, we review the different AMPAs associated with RA but not ACPAs which have been widely described in the literature.

Anti-carbamylated protein antibodies (anti-CarP)

Alongside ACPA, autoantibodies directed toward carbamylated antigens are the most studied AMPAs. Carbamylation is a chemical post-translational modification catalysed by cyanide where a lysine is transformed into a homocitrulline (47,48). Under physiological condition cyanide is expressed at low level but it rises during certain conditions such as smoking and inflammation, consequently also carbamylation increases (49). The final product of carbamylation is homocitrulline which is structurally similar to citrulline which has only one CH₂ group less compared to homocitrulline. Anti-CarP autoantibodies have been observed in around 45% of early RA patients, mostly ACPA-positive, but they can be also found in ACPA-negative patients (10–20%) (50). This observation suggests that ACPA and anti-CarP are two different classes of autoantibodies despite sharing a similar structure. Although some degree of cross-reactivity between ACPA and anti-CarP autoantibodies exist, recent finding have

shown that these are two distinct group of autoantibodies (51,52). Anti-CarP antibodies can be found in the sera of RA patients many years before the onset of the disease, similarly to ACPAs and RF (53,54). Their presence in baseline samples have been shown to be associated with future development of the disease in arthralgia patients (ACPA- and RF-negative) and with radiological progression in ACPA-negative patients (50,53,55-57). Current studies on genetic risk factors have revealed that these antibodies are not associated with HLA SEs alleles but blandly only with HLA-DRB1*03 which is linked to ACPA-negative patients (58). Finally, the precise autoantigen(s) recognised by anti-CarP autoantibodies is unknown. At the moment, immune-assays to detect the presence of these autoantibodies use fetal calf serum which contains a mix of carbamylated antigens. Therefore, the nature and localization of carbamylated antigens in the RA synovium still need to be explored in order to allow a better and more precise identification of anti-CarP antibodies in RA patients.

Anti-hinge antibodies

RA patient synovial fluid and tissue (59) are characterised by an increase level of endogenous proteases such as matrix metalloproteases (MMPs) responsible for degrading matrix proteins, thus causing tissue damage. MMPs can also cleave IgG molecules generating F(ab')₂ fragments. The result is the generation of new epitopes composed of C-terminal amino acid residues (48,60). Autoantibodies towards these new epitopes, known as anti-hinge antibodies, are increased in RA sera compared to healthy donors (61). However, their biological function is still unclear. It has been proposed that these autoantibodies could restore the effector function of F(ab')₂ fragments which can still bind their own antigen and form immune complexes but not bind complement and Fc receptor (48). Interestingly, a subset of RA patients has shown the presence of anti-hinge antibodies specifically directed towards the IgG4 hinge and this was correlated with the presence of RF and ACPA. Anti-IgG4-hinge antibodies were shown to be able to restore C4b complement deposition by IgG4 F(ab')₂ fragments (62). Therefore, anti-IgG4-hinge antibodies could have a role in the RA inflammatory process in a subset of RA patients.

Anti-acetylated protein antibodies

A new group of AMPAs recently discovered in RA are anti-

acetylated protein antibodies. In particular, Juarez and colleagues have shown that around 40% of RA patients ACPA-positive are characterised by the presence of anti-acetylated vimentin antibodies (63). Acetylation is an enzymatic post-translational modification of lysine which happens in humans and bacteria. In this reaction acetyl groups are added to free amines of lysine residues (64). It has been suggested that this new class of AMPAs could provide a new understanding of the pathophysiology of RA linking microbiome dysbiosis and development of autoimmunity (63,65).

Anti-malondialdehyde-acetaldehyde adducts (MAA) antibody

A less characterised class of AMPAs associated with RA is represented by MAA antibodies. Lipid peroxidation and cell damage can lead to the formation of different protein adducts which promote pro-inflammatory responses. One of this product is malondialdehyde (MDA) which spontaneously breaks down to form acetaldehyde (AA) (66). Both MDA and AA can react to produce MAA adducts involving lysine; MAA are stable ring structure and highly immunogenic (66-69). Thiele and colleagues recently showed the presence of MAA adducts in RA but not osteoarthritis synovial tissue and found in increased titer of anti-MAA antibodies in the circulation of RA patients (70). The presence of these antibodies was associated mainly with ACPA and RF. Although, anti-MAA antibodies were also observed in ACPA-negative patients. The disease specificity of these antibodies is still unclear, thus more studies are needed in order to characterise better this group of AMPAs.

Conclusions

In the last few years, several studies have shed more light into the pathophysiology of RA offering new views on how the disease develops. This has been achieved by increasing studies on the genetic and environmental risk factors in RA and recently on the effect of microbiome. Moreover, a better understanding of the role of AMPAs, which include not only ACPAs, has given a better overview of this heterogeneous disease. However, it is still unclear whether these autoantibodies could contribute to disease pathogenesis. Although several progresses have been done, more studies are needed in order to enhance the understanding of the pathophysiology of RA, thus achieving better therapy and ultimately preventing the disease.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/jlpm.2018.06.03>). The authors have no conflicts of interest to declare.

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